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Effects of Adjuvants on the Performance of a Novel Powdery Mildew Fungicide, 1-(4-Chlorobenzyl)-4-phenylpiperidine

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Abstract: The effects of adjuvants on the performance of a dispersible concentrate formulation (DC**I**) of a novel powdery mildew fungicide, 1-(4-chlorobenzyl)-4-phenylpiperidine (**I**) were investigated. The method involved assessment, under glasshouse conditions, of the therapeutic (curative) control of infections of powdery mildew (*Erysiphe graminis* DC f.sp. *hordei* Marchal) on barley (*Hordeum vulgare*, L.) eight to nine days after spray application to plants that had been inoculated one day prior to spraying. The results from the first trial showed that marked improvements (~five-fold) in the performance of DC**I** could be obtained by the spray tank addition of some types of surfactant adjuvants and a series of further trials investigating a wide range of adjuvants was conducted. Nonylphenol, alkylamine and alcohol ethoxylates varying in mean ethylene oxide content between 5 and 20 moles were highly effective. There were indications that optimum performance enhancements were obtained with these surfactants containing between 5 and 10 moles ethylene oxide. Lower enhancements, sometimes only marginal, were obtained from trisiloxane, phosphate ester, propylene oxide, alkylamine-propylene oxide and castor oil ethoxylates and also alkyl polyglucoside biosurfactants. Negligible adjuvant enhancements were observed with emulsifiable paraffinic/naphthenic and rape seed oils, though slightly better enhancements were seen with an emulsifiable transmethylated rape seed oil and, interestingly, larger enhancements with an emulsifiable lipophilic alcohol. A final trial identified the alcohol ethoxylate, 'Dobanol' 91-6, as the most effective adjuvant and that its optimum application rate under glasshouse conditions was 250 g ha^{-1}. This information will be used to guide the design of field trials.

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1 INTRODUCTION

1-(4-Chlorobenzyl)-4-phenylpiperidine (**I**) is a novel, potent, foliage-applied fungicide which controls the powdery mildews (Ascomycetes, Erysiphales and Erysiphaceae) *via* inhibition of the Δ8–Δ7 isomerisation step in sterol synthesis, a mode of action similar to that of morpholine fungicides.[1]

As with any foliage-applied crop-protection agent it is important to investigate the possibility of enhancement of performance that may be achieved by the use of adjuvants, such as surfactants and emulsifiable oils. These substances can aid spray deposition and facilitate penetration of the crop-protection agent through leaf cuticle barriers and thereby increase its concentration in the vicinity of the biochemical target over those achieved by formulations containing no adjuvants.[2–4] This leads to reduced application rates of the agent and a consequent improvement in its cost performance and

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a reduced loading in the environment. A research programme was devised to investigate the abilities of surfactant and emulsifiable oil adjuvants to influence the performance of a dispersible concentrate formulation of **I** in the therapeutic (curative) control of one-day-old infections of powdery mildew (*Erysiphe graminis* DC f.sp. *hordei* Marchal) on barley (*Hordeum vulgare* L.). The adjuvants included alcohol, nonylphenol, alkylamine, phosphate ester, propylene oxide and trisiloxane ethoxylates, varying systematically in the degree of ethoxylation where possible, a limited range of alkyl polyglucosides and emulsifiable paraffinic, lipid alcohol and lipid ester and vegetable oils. The style of the investigation followed that of previous, recent studies with other types of fungicides and plant varieties.[5–8] A final trial was designed to examine the effect of application rate of adjuvant upon the performance enhancement of two of the most active adjuvants to give information for the design of field trials.

2 EXPERIMENTAL

2.1 Materials

A dispersible concentrate (**I**, 200 g; emulsifier, 100 g; mixed solvents, to 1 litre, DC**I**) of **I** was supplied by the Formulation Department of Shell Forschung, Schwabenheim.

Surfactant and emulsifiable oil adjuvants were obtained as follows: C_9/C_{11} alcohol, 'Dobanol' 91, C_9/C_{11}, C_{12}/C_{13}, C_{14}/C_{15} alcohol ethoxylates, 'Dobanol' 91-2.5, 91-6, 91-8, 'Dobanol' 23-3, 'Dobanol' 45-7 containing 2·5, 6, 8, 3 and 7 moles of ethylene oxide respectively, paraffinic/naphthenic oil, HVI 60 from Shell Chemicals UK, Carrington, UK; C_{12}/C_{14} alcohol ethoxylates, 'Genapol' C050, C100, C200 containing 5, 10, 20 moles of ethylene oxide respectively, C_{12}/C_{14} alkylamine ethoxylates, 'Genamin' C050, C100, C200, containing 5, 10, 20 moles of ethylene oxide respectively, nonylphenol ethoxylates, 'Arkopal' N060, N100, N150 containing 6, 10, 15 moles of ethylene oxide respectively from Hoechst AG, Frankfurt, Germany; alkyl phosphate ester 'Gafac' RS710 from Gaf Corporation, New York, USA; trisiloxane ethoxylate/propoxylate, 'Silwet' L77 from Witco Corporation, OSi Specialities Group, Connecticut, USA; emulsifiable rape seed oil, 'Atplus' 412, alkyl polyglucosides, 'Atplus' 453, 450, 460 from ICI Specialty Chemicals, Kortenburg, Belgium; transmethylated rape seed oil, Estorob 926-67 from Robbe Chemical Co., Compiegne, France; alkylamine/propylene oxide ethoxylate, 'Armoblen' 557 from Akzo Chemie BV, Amersfoort, The Netherlands; castor oil ethoxylate containing 35 moles of ethylene oxide from Tensia SA, Liege, Belgium; propylene/ethylene oxide condensate, 'Pluronic' F-127 from BASF AG, Ludwigshafen, Germany.

The paraffinic/naphthenic oil, HVI 60, the lipophilic alcohol, 'Dobanol' 91, and the transmethylated rape seed oil, 'Estorob' 926-67, were each mixed with an emulsifier, 'Emulsogen' M (Hoechst AG, Frankfurt), in a ratio of nine parts oil to one part 'Emulsogen' M (by volume) and designated by the suffix E.

2.2 Plant propagation and inoculation procedure

Barley seed (*H. vulgare*, cv. Golden Promise) was sown into pots (70 × 70 × 80 mm depth) containing sterilised loam at a rate to give 12–15 plants/pot. The pots were transferred to glasshouse conditions (temperature, 20–25°C; relative humidity, 70–90%; lighting, 16 h photoperiod to natural daylight supplemented by mercury vapour/sodium lights giving $\sim$200 μmol m^{-2} s^{-1}) and watered daily by overhead irrigation. After six to eight days, the seeds had germinated and grown to the expanded first leaf stage (growth stage 11). This foliage was inoculated with powdery mildew (*E. graminis* f.sp. *hordei*) using dry inoculum from sporulating, diseased plants 24 h before spraying. These inoculated plants were watered only by sub-irrigation during this period.

2.3 Preparation of spray solutions and spraying procedure

Solutions were prepared by dispersing volumes of DC**I** in tap water (250 ml) as follows: Trial 1–12·5, 25, 50 and 100 μl; Trials 2, 3, 4–25, 50, 100 and 200 μl, Trials 5, 6–50, 100, 200 and 400 μl. In trials 1–5, comparing adjuvant effectiveness, a volume (20 ml) of each formulation dispersion was diluted with an equal volume of either water or solution containing 0·5 g adjuvant (also 0·25 g in Trial 5) in 200 ml tap water. In Trial 6, a volume (20 ml) of formulation dispersion was diluted with an equal volume of either water or a range of adjuvant solutions containing 0·16, 0·32 and 0·63 g adjuvant in 250 ml tap water. In all cases, these dilutions were sprayed onto quadruplicate pots of inoculated barley plants using a laboratory track sprayer equipped with an 8002E hydraulic nozzle (Spraying Systems Co., Illinois) operating at 276 kPa to give a volume rate of spray deposition of 400 litre ha^{-1}. At this volume rate, application rates of active ingredient and adjuvant were as indicated in Tables 1–6. Control plants sprayed only with water or, in some trials, with diluted adjuvant solutions were also included.

2.4 Plant treatment and assessment

After spraying, the plants were returned to the glasshouse conditions as given above except that they were placed on irrigation matting in a randomised array and watered daily by sub-irrigation only. Establishment of disease was assessed eight to nine days after spraying by

TABLE 1
Effect of Adjuvants on the Therapeutic Control of Powdery Mildew on Barley by 1-(4-Chlorobenzyl)-4-phenylpiperidine DC Formulation

Adjuvant (*500 g ha*$^{-1}$)	*Infection score*[a] *Application rate of* ***I*** (*g AI ha*$^{-1}$)					*Calculated* ED_{90} *value*[b] (*g AI ha*$^{-1}$)	*95% Confidence limits* (*g AI ha*$^{-1}$)
	0	*2*	*4*	*8*	*16*		
None	9·0	9·0	9·0	8·9	8·1	145a	113 186
'Genapol' C050	8·9	9·0	8·0	7·6	5·3	56b	45 70
'Genapol' C100	8·9	8·5	8·3	6·0	2·5	33de	28 40
'Genapol' C200	9·0	8·5	8·3	6·0	2·5	35cde	29 42
'Dobanol' 91-6	9·0	8·4	7·5	5·3	3·0	28e	24 32
'Genamin' C050	9·0	8·6	7·6	6·0	2·0	28e	23 34
'Gafac' RS710	9·0	9·0	8·8	7·6	4·5	55bc	36 59
'Silwet' L77	9·0	9·0	8·3	7·3	4·3	46bcd	36 59

[a] Scale 0–9; 0 = no infection, 9 = complete infection.
[b] Values with the same letter are not significantly different at 95% confidence level.

TABLE 2
Effect of Adjuvants on the Therapeutic Control of Powdery Mildew on Barley by 1-(4-Chlorobenzyl)-4-phenylpiperidine DC Formulation

Adjuvant (*500 g ha*$^{-1}$)	*Infection score*[a] *Application rate of* ***I*** (*g AI h*$^{-1}$)				*Calculated* ED_{90} *value*[b] (*g AI ha*$^{-1}$)	*95% Confidence limits* (*g AI ha*$^{-1}$)
	4	*8*	*16*	*32*		
None	7·1	6·4	6·4	5·5	130a	92 186
'Dobanol' 91E	6·5	6·0	2·9	1·3	39bc	30 49
'Dobanol' 91-2.5	6·1	5·3	2·9	0·7	33bc	26 41
'Dobanol' 91-6	5·4	3·0	1·3	0·6	20d	17 23
'Dobanol' 23-3	6·8	6·3	3·5	1·4	44b	35 55
'Dobanol' 45-7	5·5	4·8	0·9	0·2	18d	13 25
'Arkopal' N060	6·5	6·1	4·9	0·9	44b	33 58
'Arkopal' N100	6·1	5·0	1·5	0·6	25cd	20 31
'Arkopal' N150	6·1	5·0	0·7	0·7	25cd	20 32

[a] Scale 0–9; 0 = no infection, 9 = complete infection; infection score on plants sprayed only with water = 8·0.
[b] Values with the same letter are not significantly different at 95% confidence level.

TABLE 3
Effect of Adjuvants on the Therapeutic Control of Powdery Mildew on Barley by 1-(4-Chlorobenzyl)-4-phenylpiperidine DC Formulation

Adjuvant (*500 g ha*$^{-1}$)	*Infection score*[a] *Application rate of* ***I*** (*g AI ha*$^{-1}$)				*Calculated* ED_{90} *value*[b] (*g AI ha*$^{-1}$)	*95% Confidence limits* (*g AI ha*$^{-1}$)
	4	*8*	*16*	*32*		
None	8·6	8·1	7·5	6·0	185a	147 234
'Dobanol' 91-6	7·0	5·3	3·0	0·7	42cd	38 46
'Dobanol' 91-8	7·0	5·5	3·3	0·5	44cd	38 51
'Dobanol' 25-7	7·3	6·0	3·0	0·5	41cd	34 50
'Dobanol' 25-9	7·0	6·0	4·0	0·7	51c	44 58
'HVI 60'E	8·4	7·8	7·0	4·5	144a	122 171
'Estorob' 926-67E	7·5	7·5	5·5	3·8	102b	86 121
'Atplus' 412	8·5	8·3	6·3	5·9	148ab	113 195
'Genamin' C020	8·1	7·5	5·8	2·0	77bc	62 97
'Genamin' C100	6·9	5·6	2·8	0·4	32d	27 39

[a] Scale 0–9; 0 = no infection, 9 = complete infection; infection score on plants sprayed only with water = 9.
[b] Values with the same letter are not significantly different at 95% confidence level.

visual assessment of the area of inoculated leaf tissue covered by sporulating lesions on a linear scale of 0–9, where 0 = no lesions, 9 = complete cover with lesions. The mean values of the scores for the quadruplicate pots of plants for each treatment of **I** are given in Tables 1–6. The results obtained were also subjected to statistical analysis using a logistic dose-response model using the procedure PROC NLIN from the statistical package SAS[9] on an IBM 3090 computer, to give estimates of 90% control (ED_{90} values) and associated 95% confidence intervals.

3 RESULTS AND DISCUSSION

The results from the first trial demonstrated that the glasshouse performance of DC**I** could be markedly improved by the incorporation of adjuvants (Table 1). A series of C_{12}–C_{14} alcohol ethoxylates ('Genapol' C series) varying in ethylene oxide content between 5 and 20 moles gave substantial reductions in the ED_{90} values for **I**, with some indications that there was a structure-activity enhancement correlation slightly favouring intermediate (10 moles) to high (20 moles) ethylene content. This observation was in accord with a recently proposed hypothesis that for compounds of intermediate lipophilicity (**I**, log K_{OW} = 1·8 at pH 7) the range of ethylene oxide content for optimum surfactant-induced foliar penetration of a pesticide could be wide.[10,11] An alcohol ethoxylate with a slightly shorter aliphatic group (C_9–C_{11}, 'Dobanol' 91-6) with 6 moles of ethylene oxide was very effective, as was the C_{12}–C_{14} alkylamine ethoxylate ('Genamin' C050) with 5 moles ethylene oxide (Table 1). The alkyl phosphate ester ('Gafac' RS710) and the trisiloxane ethoxylate ('Silwet' L77) were effective but significantly less so than either 'Dobanol' 91-6 or 'Genamin' C050. There was no direct fungicidal activity from any of the surfactants when applied alone (Table 1). The enhancements of performance of DC**I** could therefore be attributed to improvements in spray deposition[12,13] and either foliar penetration[10,11,14] and/or direct fungal penetration of **I** as induced by the surfactants.

The results gave encouragement to broaden the investigation by examining a wider range of surfactants and also to include some emulsifiable oils. Several members of the range of 'Dobanol' alcohol ethoxylates, including the unethoxylated emulsifiable lipophilic alcohol ('Dobanol' 91E) all proved highly effective, again with an indication that the intermediate ethylene oxide surfactants ('Dobanols' 91-6 and 45-7) were slightly more effective than their lower ethylene oxide oligomers (Table 2). A series of nonylphenol ethoxylates

TABLE 4
Effect of Adjuvants on the Therapeutic Control of Powdery Mildew on Barley by 1-(4-Chlorobenzyl)-4-phenylpiperidine DC Formulation

Adjuvant (*500 g ha*$^{-1}$)	*Infection score*[a] *Application rate of* ***I*** (*g AI ha*$^{-1}$)				*Calculated* ED_{90} *value*[b] (*g AI ha*$^{-1}$)	*95% Confidence limits* (*g AI ha*$^{-1}$)
	4	*8*	*16*	*32*		
None	9·0	9·0	8·7	8·0	267a	125 570
'Dobanol' 91-6	8·3	6·8	5·8	2·1	56bc	46 69
'Genamin' C100	8·1	7·6	5·3	1·1	48c	39 59
'Armoblen' 557	8·0	6·0	5·5	1·9	52c	42 63
'Tensiofix' D120	8·8	7·8	5·5	4·8	89b	69 115
'Pluronic' F127	9·0	8·9	6·8	3·5	82b	64 104

[a] Scale 0–9; 0 = no infection, 9 = complete infection; infection score on plants sprayed only with water = 8·8.
[b] Values with the same letter are not significantly different at 95% confidence level.

TABLE 5
Effect of Adjuvants on the Therapeutic Control of Powdery Mildew on Barley by 1-(4-Chlorobenzyl)-4-phenylpiperidine DC Formulation

Adjuvant		*Infection score*[a] *Application rate of* ***I*** (*g AI ha*$^{-1}$)					*Calculated* ED_{90} *value*[b] (*g AI ha*$^{-1}$)	*95% Confidence limits* (*g AI ha*$^{-1}$)
Type	*Application rate* (*g ha*$^{-1}$)	*0*	*2*	*4*	*8*	*16*		
None	—	9·0	8·6	8·1	7·0	5·6	186a	169 205
'Genamin'	250	9·0	6·9	5·5	2·1	0·3	49cd	45 53
C050	500	8·9	6·0	4·1	0·9	0·4	35e	33 38
'Genamin'	250	8·9	7·3	4·5	2·1	0·4	50cd	45 54
C100	500	8·6	6·5	4·8	1·5	0·2	42de	37 47
'Genamin'	250	8·8	7·4	6·1	2·8	0·6	57bc	49 68
C200	500	8·9	7·0	4·8	2·5	0·4	47cd	43 51
'Armoblen'	250	8·9	6·6	6·4	2·9	1·5	75b	67 84
557	500	8·9	7·0	4·8	2·5	0·2	42de	37 48
'Atplus' 435	500	9·0	7·6	5·9	2·9	0·9	67b	62 73
'Atplus' 450	500	8·9	7·9	5·9	3·8	0·5	67b	59 75
'Atplus' 460	500	8·9	7·8	5·5	3·6	0·8	64b	57 73

[a] Scale 0–9; 0 = no infection, 9 = complete infection.
[b] Values with the same letter are not significantly different at 95% confidence level.

TABLE 6
Comparison of the Effects of 'Dobanol' 91-6 and 'Genamin' C050 on the Performance of a DC Formulation of 1-(4-Chlorobenzyl)-4-phenylpiperidine against Powdery Mildew on Barley

Adjuvant		*Infection score*[a] *Application rate of* ***I*** *(g IA ha*$^{-1}$*)*				*Calculated* ED_{90} *value*[b]	*95% Confidence limits*
Type	*Application rate (g ha*$^{-1}$*)*	*8*	*16*	*32*	*64*	*(g AI ha*$^{-1}$*)*	*(g AI ha*$^{-1}$*)*
None	—	8·1	6·8	6·6	4·8	228a	181 287
	125	7·3	5·3	2·1	1·1	71b	60 85
'Dobanol'	250	6·9	3·4	1·6	0·5	47c	40 55
91-6	500	6·3	3·5	3·8	0·6	50c	44 55
	125	7·1	5·0	3·8	1·5	98b	84 115
'Genamin'	250	5·9	3·8	2·1	0·7	52c	46 60
C050	500	5·8	3·8	2·0	0·7	53c	47 59

[a] Scale 0–9; 0 = no infection, 9 = complete infection; infection score on plants sprayed only with water = 8·9.
[b] Values with the same letter are not significantly different at 95% confidence level.

('Arkopals'), varying between 6 and 15 moles of ethylene oxide, were all also highly effective, with structure-activity enhancement trends again favouring intermediate ethylene oxide contents (Table 2), as observed with the alcohol ethoxylates.

Examination of further members of the range of 'Dobanol' alcohol ethoxylates confirmed that they were all of similar high effectiveness (Table 3). On the other hand, the emulsifiable paraffinic/naphthenic oil (HVI 60E) and rape seed oil ('Atplus' 412) produced little improvement for **I**, while the emulsifiable trans-methylated rape seed (Estorob 926.67E) gave some small effects (Table 3). These observations were interesting in that, in the previous trial, an emulsifiable lipophilic alcohol ('Dobanol' 91E) had proved quite effective (Table 2). There seemed to be an order of effectiveness that followed the order of lipophilicity inversely through this range of oils, i.e. lipophilic alcohol > lipophilic ester > paraffinic/naphthenic mixture.

Also included in this trial were two alkylamine ethoxylates, 'Genamin' C020, C100, containing 2 and 10 moles of ethylene oxide, respectively. Both were effective but, as with the alcohol ethoxylates, the oligomer with the intermediate ethylene oxide content, 'Genamin' C100, was significantly more effective than that with the very low ethylene oxide content, confirming the trends seen with both alcohol and nonylphenol ethoxylates.

In a fourth trial, the performance enhancement capabilities of three further types of surfactant were compared with those of 'Dobanol' 91-6 and 'Genamin' C100, two of the most efficaceous adjuvants found in the first three trials. The alkylamine/propylene oxide ethoxylate ('Armoblen' 557) was of equal effectiveness to 'Dobanol' 91-6 and 'Genamin' C100, while the castor oil ethoxylate ('Tensiofix' D120) and the propylene oxide/ethylene oxide condensate ('Pluronic' F127) were of lower efficacies, the differences being significant when compared with that observed for 'Genamin' C100 (Table 4). Further examination of the 'Genamin' series containing between 5 and 20 moles of ethylene oxide, at two adjuvant application rates, showed that they were all highly effective, being slightly more so at 500 g ha^{-1} than at 250 g ha^{-1} with each surfactant. In this series of alkylamine ethoxylates the surfactant with 5 moles of ethylene oxide ('Genamin' C050) was marginally better than the other two (Table 5). It was also more effective than 'Armoblen' 557, especially at the 250 g ha^{-1} application rate, and significantly more than the alkyl polyglucosides, 'Atplus' 435, 450 and 460.

These series of trials, examining a number of different types of adjuvant for enhancing the performance of DC**I**, provided data which allowed many of them to be rejected from further consideration. However, three types of surfactant, nonylphenol, alcohol and alkylamine ethoxylates, gave high enhancements from more than one member of each type and were difficult to differentiate on performance grounds. Other considerations, such as ease of incorporation into formulations, possible future regulatory constraints and cost, were taken into account and two surfactants, 'Dobanol' 91-6 and 'Genamin' C050, were selected for final comparison

in a trial also designed to identify the optimum application rate of the adjuvant. The results obtained (Table 6) confirmed that these two surfactants were highly efficaceous adjuvants, giving very similar performance enhancements. Although not statistically different, 'Dobanol' 91-6 gave enhancements marginally greater at each of the adjuvant application rates and on this slender evidence and cost considerations it was selected in preference to 'Genamin' C050 for further formulation development and field trial evaluation of **I**. Its optimum application rate in these glasshouse trials was 250 g ha^{-1}, with no further enhancement of DC**I** being observed at higher rates of application (Table 6); this information was used to design a field trials programme.

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